

## *Recommended Methods of Rodent Identification*

Individual animal identification is important for animal colony management (genotype identification, to track breeding crosses, etc.), for animal health / medical records, and for research data. There are several methods available for identifying rodents, including ear notches, ear punches, ear tags, tattoos of the toes/feet or tail, and subcutaneous transponders. Toe-clipping should be used only when no other individual identification method is feasible, and a strong justification is required. Each method of identification used must be described in the animal use protocol.

Please consult a Veterinary Resources Veterinarian as necessary to identify the most appropriate method of identification to ensure animal welfare while accomplishing your research objectives. Training is available upon request. Please contact Veterinary Resources at 410-706-3540.

Identification Method	Anesthesia Required?	Description	Notes / Considerations
Indelible (permanent) marker	No	Animal is restrained ( <i>indicate method</i> ) and marker is applied to the tail or hair. Markings are re-applied as needed.	<ul style="list-style-type: none"> <li>➤ This method can be used to write on the tail or hair coat, can be used in all ages.</li> <li>➤ These markings are only temporary and will be removed during the grooming process.</li> <li>➤ The markings will need to be re-applied every 2-3 days.</li> </ul>
Ear Notch	No	Animal is restrained by scruffing the dorsum high on the neck to control both the body and the head. The ear is cleaned with 70% alcohol. A small wedge-shaped notch < 3mm is created on the outer edge of the pinna (external ear) with sterile, sharp scissors. The scissors are disinfected with alcohol and allowed to dry between animals. Pressure with a sterile gauze will be applied to achieve hemostasis if bleeding occurs.	<ul style="list-style-type: none"> <li>➤ Simple, inexpensive, easy to read, can be done at 2 weeks of age or older without anesthesia, tissue can be used for genotyping.</li> <li>➤ Notches may be placed on both ears in a pattern that correlates to a numbering system.</li> <li>➤ May be difficult to achieve a precise and extensive numbering system.</li> <li>➤ If not done correctly, the tissue may grow back.</li> </ul>

Identification Method	Anesthesia Required?	Description	Notes / Considerations
Ear Punch	No	Ear punch is disinfected with 70% alcohol. Animal is restrained by scruffing the dorsum high on the neck to control both the body and the head. The ear is cleaned with 70% alcohol. Ear punch instrument is used to puncture the pinna (external ear). Punches (2 mm diameter) are only made on the outer edge or in the middle of the pinna. Pressure with a sterile gauze will be applied to achieve hemostasis if bleeding occurs.	<ul style="list-style-type: none"> <li>➤ Sharp commercial punch devices should be used for this procedure.</li> <li>➤ Simple, inexpensive, easy to read, can be done at 2 weeks of age or older without anesthesia, tissue can be used for genotyping.</li> <li>➤ Punches may be placed on both ears in a pattern that correlates to a numbering system.</li> <li>➤ Do not punch too close to the head where the cartilage is thicker and more blood vessels are present, because it is painful.</li> <li>➤ May be difficult to achieve a precise and extensive numbering system.</li> <li>➤ If not done correctly, the tissue may grow back.</li> <li>➤ Punch instruments may get out of alignment causing pain and tissue damage by poor cutting action. If any drag or catching is noticed, the punch must be discarded.</li> </ul>
Ear Tag	No	Ear tag and tag applicator are disinfected with 70% alcohol. Animal is restrained by scruffing the dorsum high on the neck to control both the body and the head. The ear is cleaned with 70% alcohol. Ear tag is applied to the middle lower pinna. Pressure with a sterile gauze will be applied to achieve hemostasis if bleeding occurs.	<ul style="list-style-type: none"> <li>➤ Ear tags can be placed starting at ~2 weeks of age.</li> <li>➤ Extensive numbering system, up to 4 digits may be achieved.</li> <li>➤ Ear tags can be sterilized.</li> <li>➤ If not placed correctly, the tag will be ripped out by the rodent or fall out on its own.</li> <li>➤ Numbers are very small and difficult to read.</li> <li>➤ Some animals have tissue reactions to the metal resulting in crusting and thickening of the cartilage.</li> <li>➤ Cannot be used in small neonates.</li> </ul>
Toe / Tail Tattoo (using 25-27G needle)	No	Animal is restrained ( <i>indicate method</i> ). The toe / tail is disinfected with 70% alcohol. A sterile needle (25-27G) is dipped in tattoo ink (i.e. India ink, commercially available tattoo paste) then used to puncture the skin leaving small dot(s) of ink. Excess ink is removed with a sterile gauze and pressure is applied to achieve hemostasis if bleeding occurs.	<ul style="list-style-type: none"> <li>➤ Can be done at any age without anesthesia.</li> <li>➤ Extensive numbering system may be achieved.</li> <li>➤ Identification is permanent.</li> <li>➤ May be technically challenging.</li> <li>➤ Numbering system may be difficult to read.</li> <li>➤ Ink may stain draining lymph node.</li> </ul>

Identification Method	Anesthesia Required?	Description	Notes / Considerations
Tail Tattoo (using commercial micro-tattoo system)	No	Animal is restrained ( <i>indicate method</i> ). The tail is disinfected with 70% alcohol. A tattoo machine with a fresh needle is used. Needle is disinfected with 70% alcohol between uses and replaced when dull. The needle is dipped into tattoo ink then applied with firm pressure to the surface of the tail. Excess ink is removed with a sterile gauze and pressure is applied to achieve hemostasis if bleeding occurs. Triple antibiotic ointment may be applied to promote healing.	<ul style="list-style-type: none"> <li>➤ Can be done at any age without anesthesia.</li> <li>➤ Extensive numbering system may be achieved.</li> <li>➤ Tattoo oil or tincture of green may be used to enhance ink uptake.</li> <li>➤ Identification is permanent when done properly. If not placed correctly, the tattoo ink will be shed with the outer skin cells of the tail.</li> <li>➤ Tattooing by hand can be difficult and takes a lot of practice.</li> <li>➤ Cannot be used in small neonates.</li> <li>➤ Numbering system may be difficult to read.</li> <li>➤ Ink may stain draining lymph node.</li> </ul>
Subcutaneous transponder (e.g., microchip, RFID transponders)	Yes	First the transponder is tested to ensure that the chip reader can recognize it. Animal is anesthetized ( <i>indicate method</i> ) and implant location (intra-scapular area) is cleaned with 70% alcohol after removing the hair by a clipper with $\leq$ #40 blade. Sterile transponders are placed with a sterile trocar ( <i>indicate size</i> ) into the scruff. Veterinary tissue glue is used to close the small incision. Placement is verified by scanning the animal with the chip reader. Animal is returned to cage after anesthetic recovery and ambulating normally.	<ul style="list-style-type: none"> <li>➤ Animal must be <math>\geq</math> PND 21.</li> <li>➤ Extensive numbering system.</li> <li>➤ Permanent identification.</li> <li>➤ Some microchip systems can be re-sterilized and re-used (Kent Scientific).</li> <li>➤ Some microchips are writable and can be assigned an ID of the lab's choosing (Kent Scientific).</li> <li>➤ Microchips can be expensive; chip readers are required (prices vary by manufacturer).</li> <li>➤ Recommendations for age and size depend on manufacturer.</li> </ul>
Toe clipping ( <i>strong scientific justification required</i> )	No	Animal $\leq$ PND 7 is restrained ( <i>indicate method</i> ). The foot is disinfected with 70% alcohol. Sterile, sharp scissors are used to cut the joint between the middle and distal phalanx. Only the distal phalanx of the digit is removed (never the dew claw), and no more than two toes per extremity are removed. Pressure is applied with sterile gauze to achieve hemostasis if bleeding occurs. KWIK stop with benzocaine may also be applied to achieve hemostasis. Animal is returned to cage after bleeding subsides.	<ul style="list-style-type: none"> <li>➤ Animal must be <math>\leq</math> PND 7.</li> <li>➤ May be difficult to perform due to small size of toes.</li> <li>➤ Remove only the most distal bone of digit.</li> <li>➤ Do not remove the dew claw.</li> <li>➤ Tissue can be used for genotyping.</li> </ul>